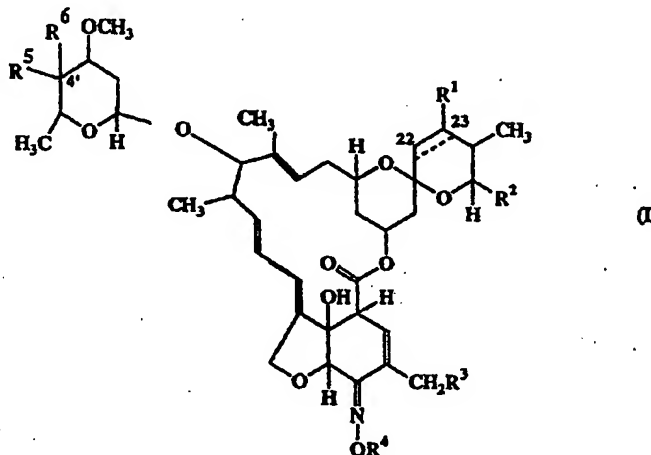




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07H 19/01, 17/08, A61K 31/70		A1	(11) International Publication Number: WO 94/15944
			(43) International Publication Date: 21 July 1994 (21.07.94)
(21) International Application Number: PCT/EP94/00095		(74) Agents: BRADBROOK, Geoffrey, William et al.; Pfizer Limited, European Patents Dept., Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).	
(22) International Filing Date: 12 January 1994 (12.01.94)			
(30) Priority Data: 9300883.7 18 January 1993 (18.01.93) GB		(81) Designated States: AU, BR, CA, CZ, FI, JP, KR, NO, NZ, PL, RU, SK, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for AT AU BE BR CA CH CZ DE DK ES FI FR GR IE IT JP KR LU MC NL NO NZ PL PT RU SE SK UA only): PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(71) Applicant (for GB only): PFIZER LIMITED [GB/GB]; Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).			
(72) Inventors; and (75) Inventors/Applicants (for US only): BISHOP, Bernard, Frank [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). PACEY, Michael, Stephen [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). PERRY, David, Austen [GB/US]; Pfizer Central Research, Eastern Point Road, Groton, CT 06340 (US).			

(54) Title: NEW ANTIPARASITIC AGENTS RELATED TO THE MILBEMYCINS AND AVERMECTINS



(57) Abstract

Antiparasitic compounds of formula (I) wherein the broken line at the 22-23 position represents an optional bond and either this bond is present and R¹ is absent or this bond is absent and R¹ is H, OH, oxo or oximino optionally substituted by C₁-C₃ alkyl group, R² is C₁-C₃ alkyl, C₂-C₃ alkenyl or C₃-C₃ cycloalkyl group, or a 3- to 6- membered heterocyclic ring containing a sulphur or oxygen atom, said ring being saturated or fully or partially unsaturated and optionally substituted by one or more C₁-C₄ alkyl groups or halogen atoms, R³ is H or OH, and R⁴ is H or a group capable of being hydrolyzed *in vivo* to yield a compound in which R⁴ is H, R⁵ is OH, optionally substituted with a group capable of being hydrolyzed *in vivo* to yield a compound in which R⁵ is OH, and R⁶ is H or C₁-C₄ alkyl or R⁶ is H and R⁵ is amino, optionally substituted with at least one group selected from C₁-C₃ alkyl and acyl groups.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

-1-

NEW ANTIPARASITIC AGENTS RELATED TO THE MILBEMYCINS AND AVERMECTINS

This invention relates to new antiparasitic agents, related to the milbemycins and avermectins and to processes for their preparation and compositions thereof.

The avermectins are a group of broad spectrum antiparasitic agents referred to previously as the C-076 compounds. They are produced by fermenting a strain of the micro-organism Streptomyces avermitilis under aerobic conditions in an aqueous nutrient medium containing inorganic salts and assimilable sources of carbon and nitrogen. The isolation and the chemical structure of the eight individual components which make up the C-076 complex is described in detail in British Patent Specification 1573955.

The C-076 complex comprises eight distinct but closely related compounds described as C-076 A1a, A1b, A2a, A2b, B1a, B1b, B2a and B2b. The "a" series of compounds refers to the natural avermectins wherein the 25-substituent is (S)-sec-butyl and the "b" series to those wherein the 25-substituent is isopropyl. The designations "A" and "B" refer to avermectins wherein the 5-substituent is methoxy or hydroxy, respectively, and the numeral "1" refers to avermectins wherein a double bond is present at the 22-23 position, and numeral "2" to avermectins lacking the 22-23 double bond and having a hydrogen at the 22-position and hydroxy at the 23 position.

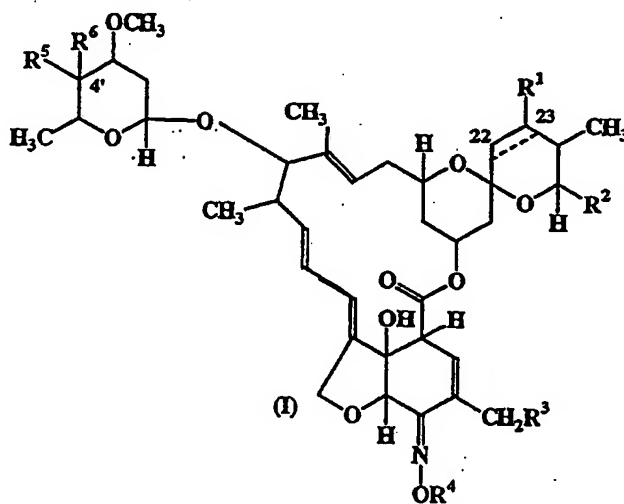
In our European Patent Applications 0214731, 0284176, 0317148, 0308145, 0340832, 0335541 and 0350187 there are described preparations of compounds related to the avermectins but having a group at the 25-position other than the isopropyl or (S)-sec-butyl groups found in the original avermectin compounds disclosed in British Patent Specification 1573955. Such compounds may be prepared by fermentation of particular strains of

Streptomyces avermitilis in the presence of organic acids or derivatives thereof. Production of such avermectins is described in Journal of Antibiotics (1991), 44, No. 3, pp 357-365.

The milbemycins form another group of related macrolides which are distinguished from the avermectins in lacking a sugar residue attached at the C-13 position. Examples of such compounds are described in UK patent 1390336, and European patent publications 170006, 254583, 334484 and 410615. In addition to these fermentation products a large number of publications describe compounds derived semisynthetically from these fermentation products many of which possess useful antiparasitic activities. Some of this chemistry is reviewed in Macrolide Antibiotics, Omura S., Ed., Academic press, New York (1984) and by Davies, H.G., Green, R.H. in Natural product Reports (1986), 3, 87-121 and in Chem. Soc. Rev., 1991, 20, 271-339.

It has been found that certain compounds synthetically derivable from known avermectins and avermectin derivatives possess unexpected beneficial biological properties.

According to one aspect of the invention there are provided compounds of formula (I):



-3-

wherein the broken line at the 22-23 position represents an optional bond and either this bond is present and R¹ is absent or this bond is absent and R¹ is H, OH, oxo or oximino optionally substituted by a C₁-C₈ alkyl group, R² is a C₁-C₈ alkyl, C₂-C₈ alkenyl or C₃-C₈ cycloalkyl group, or a 3- to 6- membered heterocyclic ring containing a sulphur or oxygen atom, said ring being saturated or fully or partially unsaturated and optionally substituted by one or more C₁-C₄ alkyl groups or halogen atoms,

R³ is H or OH, and

R⁴ is H or a group capable of being hydrolysed in vivo to yield a compound in which R⁴ is H,

R⁵ is OH, optionally substituted with a group capable of being hydrolysed in vivo to yield a compound in which R⁵ is OH.

and R⁶ is H or C₁-C₄ alkyl, or R⁶ is H and R⁵ is amino, optionally substituted with at least one group selected from C₁-C₄ alkyl and acyl (which may be alkanoyl) groups.

Unless the context otherwise requires, all alkyl and alkenyl substituents having 3 or more carbon atoms may be straight or branched-chain. The term "aryl" includes phenyl which may be substituted by at least one C₁-C₈ alkyl, hydroxy, C₁-C₈ alkoxy, halo, nitro or CF₃ group. In the present invention the term "alkyl" is intended to indicate those alkyl groups of from 1 to 8 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, pentyl, hexyl, and the like, either straight or branched chain. The term "alkanoyl" is intended to include those alkanoyl groups of from 1 to 8 carbon atoms such as formyl, acetyl, propionyl, butyryl, pentanoyl, hexanoyl, and the like.

The term "carbamoyl" is intended to indicate the group -CONR₁R₂, where R₁ and R₂ are the same or different and are H, alkyl, aryl, heteroaryl or from a 4-8 membered ring containing 1 or more O, N, or S atoms.

The above structural formula is shown without a

-4-

definitive stereochemistry. However, during the course of the synthetic procedures used to prepare such compounds, the products of such procedures can be a mixture of stereoisomers. In particular, the stereoisomers at the 4"-, 4'-, 5, 13- and 23-positions may be oriented either α - or β -representing such groups being below or above the general plane of the molecule, respectively. In each of such case both the α - and β -configurations are intended to be included within the ambit of this invention. In certain cases the term "epi" is used to distinguish the stereoisomer being of opposite configuration to the natural compound at one specific asymmetrical carbon atom.

Groups hydrolysable in vivo to yield corresponding compounds in which the group is replaced by H are well known in the pharmaceutical art in general and a wide variety of such groups are suitable for use in the compounds of this invention. Examples of such groups are C₂-C₆ alkanoyl, aroyl, carbamoyl, C₁-C₆ alkoxy carbonyl groups, and dicarboxylic acid and amino acid residues. Particular groups are identified in the Examples below. Preferred compounds are those in which R² is a straight or branched C₁-C₆ alkyl or cycloalkyl group, such as cyclohexyl, isopropyl or sec-butyl, R³ is H, and the optional bond at the 22-23 position is present or this optional bond is absent and R¹ is H or OH.

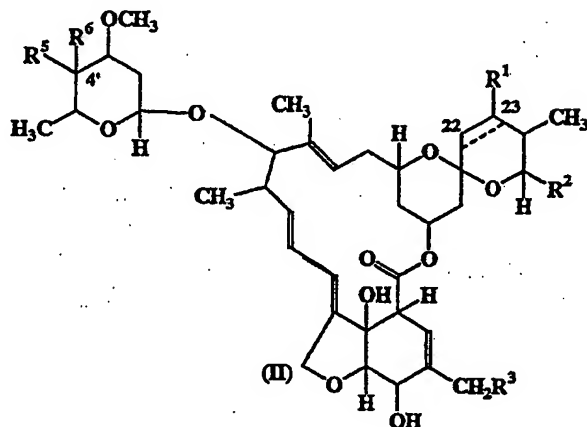
-5-

The oxime monosaccharides in which R^3 and R^4 are H are particularly preferred.

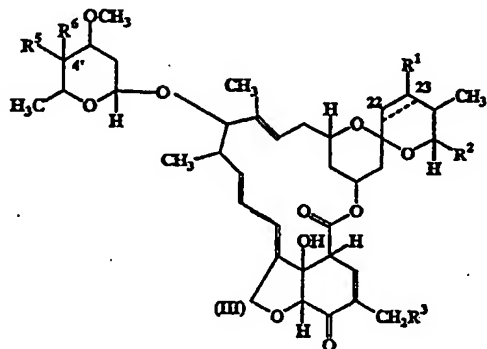
Individual compounds within the invention are described in the Examples given below.

The most preferred compound is 5-oximino-22,23-dihydro-25-cyclohexyl avermectin B1 monosaccharide.

According to another aspect of the invention, there is provided a process for preparing such a compound which comprises the steps (1) of oxidising a compound of formula (II):



wherein the broken line, R^1 , R^2 , R^3 and R^6 are as defined above and R^5 is as defined above or R^5 is α -oleandrosyl oxy and R^6 is H to yield a compound of formula (III):



-6-

and (ii) allowing the compound of formula (III) to react with a compound of formula R^4-O-NH_2 , where R^4 is as defined above and where R^5 is α -oleandrosyl oxy, hydrolysing the compound obtained to yield a compound of formula (I), and (iii) if necessary replacing group R^4 when the latter is H with said group capable of being hydrolysed in vivo to yield a compound in which R^4 is H, if necessary, the process further comprising one or more of the following steps before or after steps (i), (ii) and (iii),

(iv) substituting group R^5 when the latter is OH with said group capable of being hydrolysed in vivo to yield a compound in which R^5 is OH,

(v) oxidising group R^1 when the latter is OH to oxo,

(vi) reacting the compound obtained from step (v) with hydroxylamine optionally substituted by a C_1-C_4 alkyl group to yield a compound in which R^1 is optionally substituted oxo,

(vii) hydrogenating the compound to reduce a double bond at the 22-23 position to a single bond,

(viii) oxidising a compound in which R^3 is H to a compound in which R^3 is OH,

(ix) oxidising a compound in which R^5 is OH and R^6 is H to a compound in which R^5 is oxo and R^6 is absent, and either:

(x) reducing the compound obtained from (ix) to produce a compound in which R^5 is an epi-OH group, or

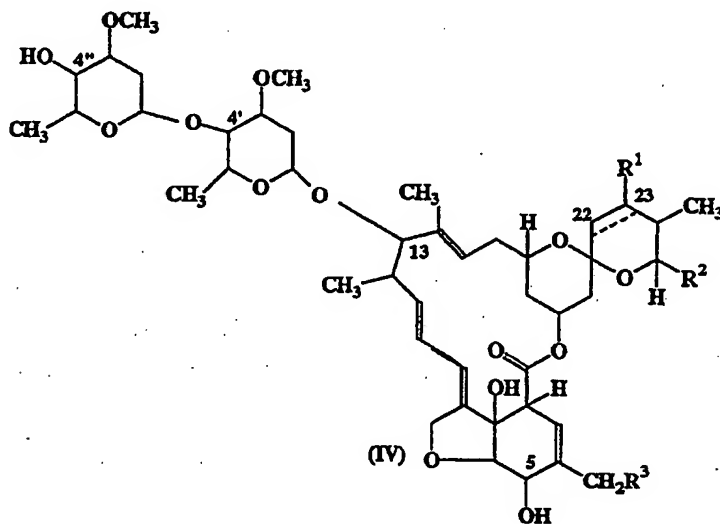
(xi) reacting the compound obtained from (ix) with a Grignard reagent to yield a compound in which R^5 is OH and R^6 is alkyl, or

(xii) subjecting the compound obtained from (ix) to reductive amination to yield a compound in which R^5 is an amino or alkylamino group, and if necessary acylating the compound obtained, any free OH groups being protected if necessary during any of the above steps.

Preparation of compounds according to the invention is discussed, by way of illustration, below.

-7-

The compounds of the present invention may be prepared starting from the compounds of formula (iv), which may themselves be prepared as described in the above-mentioned patent publications.



Compound IIa	Double bond present, R^1 absent.
Compound IIb	Double bond absent, $R^1 = H$.
Compound IIc	Double bond absent, $R^1 = OH$.

The semisynthetic modifications required to provide the compounds given by formula I may require sequential reactions at the positions -4', 4'', 4a, 13, 22, 23, 25 and 5 and the exact order in which these transformations are performed may vary. In addition, during the oxidation and certain substitution reactions described below, it is necessary to protect the 5-hydroxy group to avoid substitution or oxidation at that position. With this position protected, the reactions may be carried out

-8-

at the 4"-, or 4'-positions without affecting the remainder of the molecule. Subsequent to any of the above-described reactions, the protecting group may be removed and the unprotected product isolated. For compounds of this invention, conversion of the 5-hydroxy group to a ketone or ketoxime is preferably performed after substitution at the 4', 23 and 4a positions. The protecting group employed at the 5-position is ideally one which may be readily synthesized, will not be affected by the reactions at the 4"- and 4'-positions and may be removed without affecting any other functionality of the molecule. One preferred type of protecting group of the avermectin type of molecule is the tri-substituted silyl group, preferably the tri-loweralkyl silyl group. One especially preferred example is the tert.-butyldimethylsilyl group. The reaction preparing the protected compound is carried out by reacting the hydroxy compound with the appropriately substituted silylhalide, preferably the silylchloride in an aprotic solvent such as methylene chloride, benzene, toluene, ethyl acetate, tetrahydrofuran, dimethylformamide and the like. In order to minimise side reactions, there is included in the reaction mixture a base to react with the acid released during the course of the reaction. Preferred bases are amines such as imidazole, pyridine, or triethylamine. The base is required in amounts equimolar to the amount of hydrogen halide liberated; however, generally several equivalents of the amine are employed. The reaction is stirred at from 0°C to the reflux temperature of the reaction mixture and is complete in from 1/2 to 16 hours. The silyl group is removed by treatment of the silyl compound with anhydrous pyridine-hydrogen fluoride in tetrahydrofuran. The reaction is complete in from 3 to 24 hours at from 0 to 25°C. Alternatively, the silyl group may be removed by stirring the silylated compound in methanol catalyzed by an acid

preferably a sulfonic acid monohydrate such as p-toluenesulfonic acid monohydrate. The reaction is complete in about 1 to 12 hours at from 0 to 50°C. Compounds having a 23-hydroxyl group (or a protected derivative) may be converted to either the corresponding 22,23-dihydro compound or alternatively to the corresponding compound having a double bond at the 22-23 position using methods described in US patent 4328335. The latter compounds may also be hydrogenated to the 22,23-dihydro compounds using Wilkinson's catalyst under conditions described in US patent 4199569.

The preparation of the compounds of the invention can be accomplished by first converting the aforementioned disaccharides IIa, b and c into their corresponding monosaccharides by hydrolysis. An alternative method of preparing the monosaccharides comprises a direct fermentation procedure starting from a corresponding aglycone as described in European Patent Application 463677. The 5-hydroxy position is then protected to avoid substitution at this position so that reaction may be carried out at the 4', or 23 positions. The 4'-hydroxy is more reactive than the 23-hydroxy, hence additional suitable protection at C-4' allows selective reaction at C-23.

Alternatively, the compounds of the invention may be prepared by carrying out the above synthetic manipulations on the disaccharides IIa, b or c and finally hydrolysing them to the desired monosaccharides.

When desired, hydroxy groups may be acylated to give esters using reagent such as acid anhydrides or acid chlorides and amine bases according to general procedures known to those skilled in the art. Hydroxy groups may be converted to oxo groups by oxidation with manganese dioxide or tetrapropylammonium perruthenate. The oxo compound may be treated with hydroxylamine or an O-substituted analogue thereof to produce the corresponding oxime.

Compounds in which R³ is OH are prepared from suitably 5-protected derivatives by firstly hydroxylating the 4a methyl group using a procedure described in the Japanese Patent Laid-Open Patent Application No. 83-59988.

Compounds in which R⁵ is an amino group optionally substituted by one or more alkyl or acyl groups, may be prepared by reduction amination of a corresponding compound in which R⁵ is oxo, for example by reaction with an ammonium salt or amine salt and sodium cyanoborohydride in known manner. The amino group may then be further substituted, by acetylation, for example using acetic anhydride.

Compounds in which R³ is an epi-OH group may be made by reduction of such an oxo compound. The oxo compound may be converted to a compound in which R⁵ is OH and R⁶ is alkyl by reaction with a Grignard reagent in known manner.

-11-

The compounds of the invention are effective in treating a variety of conditions caused by endoparasites including, in particular, helminthiasis which is most frequently caused by a group of parasitic worms described as nematodes and which can cause severe economic losses in swine, sheep, horses and cattle as well as affecting domestic animals and poultry. The compounds are also effective against other nematodes which affect various species of animals including, for example:- Dirofilaria in dogs and various parasites which can infest livestock, companion animals such as cats and dogs and also humans including gastro-intestinal parasites such as Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Toxocara, Toxascaris, Trichuris, Enterobius and parasites which are found in the blood or other tissues and organs such as filarial worms and the extra intestinal stages of Strongyloides, Toxocara and Trichinella.

The compounds are also of particular value in treating ectoparasite infections including particular arthropod ectoparasites of humans, animals and birds such as ticks, mites, lice, fleas, blowfly, biting insects and migrating dipterous larvae which can affect cattle and horses.

The compounds are also insecticides active against household pests such as the cockroach, clothes moth, carpet beetle and the housefly as well as being useful against arthropod pests of stored grain and of agricultural plants such as spider mites, aphids, caterpillars and against migratory orthopterans such as locusts. We have discovered that compounds within the scope of this invention are both safe and have unexpectedly high potent systemic activity against fleas and other important arthropod parasites of cats and dogs.

-12-

The compounds of formula (I) may be administered as a formulation appropriate to the specific use envisaged and to the particular species of host animal being treated and the parasite or insect involved. The compounds may be administered by injection, either subcutaneously or intramuscularly, alternatively they may be administered orally in the form of a capsule, bolus, tablet, chewable tablet or liquid drench, or they may be administered as a topical formulation or as an implant. For topical application dip, spray, powder, dust, pour-on, spot-on, jetting fluid, shampoos, collar, tag or harness may be used. Such formulations are prepared in a conventional manner in accordance with standard veterinary practice. Thus capsules, boluses or tablets may be prepared by mixing the active ingredient with a suitable finely divided diluent or carrier, additionally containing a disintegrating agent and/or binder such as starch, lactose, talc, or magnesium stearate. A drench formulation may be prepared by dispersing the active ingredient in an aqueous solution together with dispersing or wetting agents and injectable formulations may be prepared in the form of a sterile solution or emulsion. Pour-on or spot-on formulations may be prepared by dissolving the active ingredient in an acceptable liquid carrier vehicle, such as butyl digol, liquid paraffin or non-volatile ester with or without addition of a volatile component such as isopropanol. Alternatively, pour-on, spot-on or spray formulations can be prepared by encapsulation to leave a residue of active agent on the surface of the animal. These formulations will vary with regard to the weight of active compound depending on the species of host animal to be treated, the severity and type of infection and the body weight of the host. The compounds may be administered continuously, particularly for prophylaxis, by known methods. Generally for oral, parenteral and pour-on administration a dose of from about 0.001 to 10mg per kg

-13-

of animal body weight given as a single dose or in divided doses for a period of from 1 to 5 days will be satisfactory but of course there can be instances where higher or lower dosage ranges are indicated and such are within the scope of this invention.

As an alternative the compounds may be administered with the animal feedstuff and for this purpose a concentrated feed additive or premix may be prepared for mixing with the normal animal feed.

For use as an insecticide and for treating agricultural pests the compounds are applied as sprays, dusts, pour-on formulations, emulsions and the like in accordance with standard agricultural practice.

For human use the compounds are administered as a pharmaceutically acceptable formulation in accordance with normal medical practice.

The preparation of compounds according to the invention are illustrated by the following Examples.

-14-

EXAMPLE 122,23-Dihydroavermectin Bla monosaccharide

22,23-Dihydroavermectin Bla (50g) was dissolved in a mixture of isopropanol (100ml) and sulphuric acid (1ml) and stirred at room temperature under a nitrogen atmosphere for 48 hours. The reaction mixture was poured onto crushed ice and extracted with dichloromethane (2 x 200ml). The combined extracts were washed with aqueous saturated sodium bicarbonate solution (100ml), dried over anhydrous magnesium sulphate and concentrated under vacuum to give white crystals (14g) which were removed by filtration. Mass and NMR spectra were fully consistent with the proposed structure.

The Blb analogue was obtained by an identical method starting from 22,23-dihydroavermectin Blb.

EXAMPLE 25-Oxo-22,23-dihydroavermectin Bla monosaccharide

22,23-dihydroavermectin Bla monosaccharide (14g) was dissolved in diethyl ether (200ml) and activated manganese dioxide (14g) added. The mixture was stirred at room temperature for 4 hours, filtered and evaporated to dryness under vacuum to yield the title product (11.4g) whose NMR spectrum was fully consistent with the proposed structure.

The Blb analogue was obtained by an identical method starting from 22,23-dihydroavermectin Blb monosaccharide.

-15-

EXAMPLE 35-Oximino-22,23-dihydroavermectin B1a monosaccharide

5-Oxo-22,23-dihydroavermectin B1a monosaccharide

(1g) was dissolved in dry pyridine (25ml) and hydroxylamine hydrochloride (1g) added. The stirred reaction mixture was heated under reflux for 4 hours and after cooling poured onto crushed ice and extracted with dichloromethane (2 x 50ml). The combined extracts were dried over anhydrous magnesium sulphate and evaporated under vacuum to give a crude gum (1.1g). This material was purified using high pressure liquid chromatography on a Dynamax (trade mark) column (41.4 x 250mm, 8µm, ODS-silica, Rainin) eluting with methanol - water 83:17 at 42ml per minute. Appropriate fractions were combined and evaporated to dryness to yield the title product as a white solid, melting point 180-190°C. Mass and NMR spectra were fully consistent with the proposed structure.

5-Oximino-22,23-dihydroavermectin B1b was prepared by an identical method starting from 5-oxo-22,23-dihydroavermectin B1b monosaccharide.

-16-

EXAMPLE 422,23-Dihydro-25-cyclohexylavermectin B1
monosaccharide

25-Cyclohexylavermectin B1 (9.9g) was dissolved in toluene (1 litre) and Wilkinson's catalyst (tris(triphenylphosphine)rhodium(I) chloride) (9.25g) was added. The solution was hydrogenated on a large Parr (trade mark) shaker at room temperature at 50 psi hydrogen pressure. After 3 hours the reaction vessel was depressurised and allowed to stand for 12 hours before addition of a further portion of catalyst (5g) and hydrogenated as before for a further 2 hours after which no starting material remained. The solution was filtered, evaporated to dryness under vacuum and the residue chromatographed on silica eluting with dichloromethane then dichloromethane:methanol 9:1. The crude product was then chromatographed again on silica (200g) eluting with dichloromethane:methanol 19:1 to give after evaporation of the solvent under vacuum impure 22,23-dihydro-25-cyclohexylavermectin B1 as a brown foam (10g). This material was dissolved in a mixture of isopropanol (200ml) and sulphuric acid (2ml) and the brown solution was stirred at room temperature for 15 hours then poured into a mixture of ice and water (500ml) and extracted with dichloromethane (3 x 200ml). The organic layer was washed with saturated aqueous potassium hydrogen carbonate solution (100ml), water (2 x 50ml) dried over anhydrous magnesium sulphate and evaporated under vacuum to give a crude gum which was chromatographed on silica (100g) eluting with dichloromethane then dichloromethane:ethyl acetate 2:1 to give the title compound (8.2g). Mass and NMR spectra were fully consistent with the proposed structure.

-17-

EXAMPLE 55-Oximino 22,23-dihydro-25-cyclohexylavermectin B1 monosaccharide

22,23-Dihydro-25-cyclohexylavermectin B1 monosaccharide (8.2g) was oxidized to the 5-oxo derivative using manganese dioxide in anhydrous diethyl ether according to the procedure of Example 2. The crude product was purified by chromatography on silica (50g) to give the 5-oxo compound (3.22g) as a yellow foam. This was dissolved in anhydrous pyridine (60ml) and hydroxylamine hydrochloride (3.22g) was added. After stirring for 15 hours at room temperature a further aliquot of hydroxylamine hydrochloride (3.22g) was added and the solution heated to 50°C until no starting material remained. The solution was poured into water (50ml) and extracted with diethyl ether (3 x 50ml). The organic layer was washed with water, saturated sodium chloride solution, dried over anhydrous sodium sulphate and evaporated to dryness under vacuum. The crude product was chromatographed on silica (25g) eluting with dichloromethane: ethyl acetate 4:1 and finally purified by high pressure liquid chromatography using a Dynamax (trade mark) column (41.4 x 250mm, 8µm ODS-silica, Rainin) eluting with methanol:water 9:1 at 65ml per minute. Appropriate fractions were combined and evaporated under vacuum to give the title compound (1.53g). Mass and NMR spectra were fully consistent with the proposed structure.

-18-

EXAMPLE 625-Cyclohexylavermectin B2 monosaccharide

25-Cyclohexylavermectin B2 (10g) was suspended in isopropanol (100ml) and a solution of sulphuric acid (2ml) in isopropanol (100ml) was added. After stirring at room temperature for 24 hours the clear solution was poured into ice (600g) and extracted with dichloromethane (2 x 100ml). The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in tetrachloromethane and the solution stored at 4°C. The crystals which separated slowly were periodically removed by filtration and found to be the pure title compound. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 75-Oximino-25-cyclohexylavermectin B2 monosaccharide

Using the procedures of Examples 2 and 3, 25-cyclohexylavermectin B2 monosaccharide was converted to the title compound. Mass and NMR spectra were fully consistent with the proposed structure.

-19-

EXAMPLE 825-Cyclohexylavermectin B1 monosaccharide

25-Cyclohexylavermectin B1 (20g) was dissolved in tetrahydrofuran (250ml) and a mixture of tetrahydrofuran (250ml), water (10ml) and sulphuric acid (10ml) was added. The mixture was stirred at room temperature for 15 hours then poured into a mixture of ice (500g) and water (1 l) and extracted with dichloromethane (2 x 500ml). The organic layer was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulphate and evaporated under vacuum to give a foam. This was chromatographed on silica (150g) eluting with ethyl acetate - dichloromethane 1:1 to give a crude product (13.3g). Final purification was achieved by reverse phase hplc using a Dynamax (trade mark) column (41.4 x 250mm, 8µm ODS-silica, Rainin) eluting with methanol-water 4:1 at 70ml per minute to give the pure title compound. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 95-Oximino-25-cyclohexylavermectin B1 monosaccharide

Using the procedures of Examples 2 and 3, 25-cyclohexylavermectin B1 monosaccharide was converted to the title compound. Mass and NMR spectra were fully consistent with the proposed structure.

-20-

EXAMPLE 105-O-t-Butyldimethylsilyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

22,23-dihydro-25-cyclohexyl-avermectin B1 monosaccharide (Example 4) (12.1g) and imidazole (7.2g) were dissolved in dry dimethylformamide (10ml). To this solution at room temperature was added t-butyldimethylsilyl chloride (7.9g). After 18 hours the mixture was poured into ice-water (200ml) acidified to pH2 with 2N HCl and extracted with diethyl ether (2 x 80ml). The combined extracts were washed with saturated aqueous potassium bicarbonate solution (50ml) and water (50ml), dried over anhydrous sodium sulphate and evaporated under vacuum to give a crude product (14.9g). This material was further purified by chromatography on silica (Kieselgel 60, 230-240 mesh, Merck) (300g) eluting with dichloromethane - ethyl acetate 9:1. Appropriate fractions were combined and evaporated to dryness to give the title product (8.35g). The NMR spectrum was fully consistent with the proposed structure.

EXAMPLE 115-Oxo-avermectin B1a

Avermectin B1a (2.4g) was dissolved in diethyl ether (50ml) and activated manganese dioxide (2.0g) added. The mixture was stirred at room temperature for 18 hours, filtered and evaporated to dryness under vacuum to yield the title product whose NMR spectrum was fully consistent with the proposed structure.

-21-

EXAMPLE 125-Oximino-avermectin Bla

5-Oxo-avermectin Bla (800mg) (Example 11) was dissolved in pyridine (10ml) and hydroxylamine hydrochloride (800mg) added. After stirring at room temperature for 1 hour the mixture was poured into an ice (50g) and water (50ml) mixture, acidified to pH 4 with concentrated hydrochloric acid and extracted with dichloromethane (3 x 30ml). The combined extracts were washed with water (20ml), dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure to yield a crude material (1g). This material was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (100g) eluting with dichloromethane: ethyl acetate 2:1 and finally purified by high pressure liquid chromatography using a Dynamax (trade mark) column (41.4 x 250mm, 8µm ODS-silica, Rainin) eluting with methanol:water 85:15 at 70ml per minute. Appropriate fractions were combined and evaporated under vacuum to give the title compound (290mg). Mass and NMR spectra were fully consistent with the proposed structure.

-22-

EXAMPLE 135-Oximino-avermectin B1a monosaccharide

5-Oximino-avermectin B1a (50mg) (Example 12) was dissolved in a mixture of isopropanol (1 ml) and sulphuric acid (10 µl) and stirred at room temperature under a nitrogen atmosphere for 48 hours. A saturated aqueous solution of sodium bicarbonate (1 ml) was then added and the product extracted with ethylacetate (2 x 5 ml). The combined extracts were dried over anhydrous magnesium sulphate and concentrated under vacuum. The resulting crude product (25 mg) was purified using high pressure liquid chromatography on an Ultrasphere (trade mark) column (24 x 250 mm, 5 microns, ODS-silica, Beckman) eluting with methanol: water 85:15 at 20 ml per minute. Appropriate fractions were combined, to give the title product. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 144'-O-Acetyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

5-O-t-Butyldimethylsilyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 10) (216mg) was dissolved in dichloromethane (25ml) containing pyridine (400mg). Acetic anhydride (255mg) in dichloromethane (5ml) was added slowly to this solution at room temperature and the reaction mixture was allowed to stand for 72hr. The solution was poured into water (20ml), the organic layer was washed with aqueous citric acid solution (20%, 2 x 10ml), saturated aqueous potassium bicarbonate solution (2 x 10ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum. This crude product was chromatographed on silica

(25g) eluting with dichloromethane - ethyl acetate 9:1. Appropriate fractions were combined and evaporated under vacuum to give 4'-O-acetyl-5-O-t-butyldimethylsilyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide which was dissolved in methanol (20ml) containing p-toluenesulphonic acid (230mg). After stirring at room temperature for 1 hr, saturated aqueous potassium bicarbonate solution (5ml) was added and the product extracted with diethyl ether (2 x 10ml). The combined organic extracts were dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the title product as a white powder (139mg) whose NMR spectra were fully consistent with the proposed structure. This material was used in the following example without further purification.

EXAMPLE 15

4'-O-Acetyl-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-O-Acetyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (139mg) (Example 14) was oxidised to the 5-oxo-derivative using activated manganese dioxide (140mg) in diethyl ether (20ml) according to the method described in example 2. To a solution of this 4'-O-acetyl-5-oxo-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide in a mixture of methanol - dioxan 1:1 (20ml) was added a solution of hydroxylamine hydrochloride (176mg) dissolved in water (5ml). This mixture was stirred at 40°C for 3 hr before quenching with the addition of solid potassium bicarbonate (200mg) and diethyl ether (50ml). The organic extract was washed with saturated aqueous sodium chloride solution (10ml), dried

over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product. This material was then purified by chromatography on silica (Kieselgel 60, 230-400 mesh, Merck) (10g) eluting with dichloromethane - ethyl acetate 4:1. Appropriate fractions were combined and evaporated to give the desired compound which was further purified using high pressure liquid chromatography on a Ultrasphere (trade mark) column (10 x 250mm, 5µm, ODS-silica, Beckman) eluting with acetonitrile:methanol:water 71:14:15 at 5ml per minute. Appropriate fractions were combined and evaporated to dryness to yield the title product as a white solid (46mg). Mass and NMR spectra were fully consisted with the proposed structure.

EXAMPLE 16

4'-oxo-5-O-Butyldimethylsilyl-25-cyclohexyl-22,23-dihydro avermectin B1 monosaccharide

5-O-Butyldimethylsilyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 10) (1.4g) was dissolved in dichloromethane (300ml) at room temperature together with N-methyl morpholine-N-oxide (3.14g) and tetra-n-propylammonium perruthenate (233mg). Powdered molecular sieve, 4Å (187mg) was added and the mixture stirred. After 1 hour aqueous sodium sulphite solution, (50ml, 5%) was added and the separated organic phase washed with a second portion of aqueous sodium sulphite (50ml, 5%), water (2 x 50ml), saturated aqueous sodium chloride solution (2 x 50ml) and dried over anhydrous sodium sulphate. Evaporation of the solvent under vacuum followed by chromatography on silica

(Kieselgel 60, 230-400 mesh, Merck) eluting with dichloromethane - ethyl acetate 9:1 and combination and evaporation of appropriate fractions, gave the title product as a solid (857mg). The NMR spectra were fully consistent with the proposed structure.

EXAMPLE 17

4'-epi-5-O-t-Butyldimethylsilyl-25-cyclohexyl-22,23-dihydro avermectin B1 monosaccharide

4'-oxo-5-O-t-Butyldimethylsilyl-25-cyclohexyl-22,23-dihydroa vermectin B1 monosaccharide (200mg) was dissolved in methanol (10ml) at 0°C and sodium borohydride (20mg) added portionwise with stirring. After 15 minutes the mixture was poured into water, extracted with diethyl ether (2 x 30ml) and the combined organic extract washed with water (20ml), saturated aqueous sodium chloride solution (20ml) and dried over anhydrous sodium sulphate. Evaporation of the solvent under vacuum afforded the title product as a white solid, 141mg. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 18

4'-epi-25-Cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-epi-5-O-t-Butyldimethylsilyl-22,23-dihydroavermectin B1 monosaccharide 141mg, was dissolved in methanol (20ml) containing p-toluenesulphonic acid (200mg). After 18 hours the reaction mixture was basified by adding saturated aqueous potassium bicarbonate solution (20ml) and extracted with diethyl

ether (2 x 50ml). The combined extracts were washed with water (20ml), saturated aqueous sodium chloride solution (20ml), dried over anhydrous sodium sulphate and evaporated under vacuum to yield the crude product. Chromatography of this material on silica (Kieselgel 60, 230-400 mesh, Merck) (2g) eluting with dichloromethane - ethyl acetate 4:1 and combination and evaporation under vacuum of appropriate fractions, afforded the title product (100mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 19

4'-epi-5-Oxo-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-epi-25-Cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (196mg) was oxidised with activated manganese dioxide according to the procedure described in Example 2. The NMR spectrum of the product (160mg) was fully consistent with the proposed structure.

EXAMPLE 20

4'-epi-5-Oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-epi-5-Oxo-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (160mg) was treated with hydroxylamine hydrochloride (150mg) in a methanol - dioxan - water mixture 1:1:0.5 (25ml) and the product extracted, according to the procedure described in Example 15. Purification was achieved using high pressure liquid chromatography on a Ultrasphere (trade mark) column (10 x 250mm, 5µ, ODS-silica, Beckmann) eluting with acetonitrile - methanol - water, 61:14:25 at 4ml per

-27-

minute. Appropriate fractions were combined and evaporated under vacuum to give the title product as a solid (25mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 21

4a-Hydroxy-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a solution of 25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 4) (5g) in dichloromethane (150ml) was added a solution of selenium dioxide (370mg) and t-butylhydroperoxide (70%, 3.7ml) in dichloromethane (50ml) and the whole stirred at room temperature for 48 hours. A further solution containing selenium dioxide (370mg) and t-butylhydroperoxide (70%, 3.7ml) in dichloromethane (50ml) was added and stirring continued for 24 hours. After addition of water (20ml) the reaction mixture was extracted with dichloromethane (2 x 25ml) and the combined extracts washed with 10% aqueous sodium bicarbonate solution (20ml), and water (20ml), dried over anhydrous sodium sulphate and evaporated under vacuum to yield the crude product as a yellow foam, 4.5g. This material was further purified by chromatography over silica (Kieselgel 60, 230-400 mesh, Merck) (120g) eluting with dichloromethane - methanol 97:3, taking 200ml fractions. Material eluting in fractions 21-24 was combined and evaporated under vacuum to yield the title compound as a white powder. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 224a-t-Butyldimethylsilyloxy-25-cyclohexyl-22,23-dihydroaevermectin B1 monosaccharide

4a-Hydroxy-25-cyclohexyl-22,23-dihydroaevermectin B1 monosaccharide (1.26g), triethylamine (0.2ml) and 4-dimethylaminopyridine (26mg) were dissolved by stirring in dry dichloromethane (120ml) and t-butyldimethylsilyl chloride (0.32g) added portionwise. The whole was stirred under a nitrogen atmosphere for 18 hours after which further quantities of triethylamine (0.4ml), 4-dimethylaminopyridine (252mg) and t-butyldimethylsilyl chloride (0.64g) were added and stirring continued for 3 hours. An aqueous solution of sodium bicarbonate (10%, 100ml) was added to the reaction and the organic layer separated, washed with water (100ml), dried over anhydrous sodium sulphate and evaporated under vacuum to give the crude product. This material was purified by chromatography on silica (Kieselgel 60, 230-400 mesh, Merck) (120g) eluting with dichloromethane - methanol, 98:2 collecting 130ml fractions. Material eluting in fractions 12-14 were combined and evaporated under vacuum to yield the title compound as a white solid, (1.1g). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 234a-t-Butyldimethylsilyloxy-5-oxo-25-cyclohexyl-22,23-dihydroaevermectin B1 monosaccharide

4a-t-Butyldimethylsilyloxy-25-cyclohexyl-22,23-dihydroaevermectin B1 monosaccharide (1.1g) was oxidised with activated manganese dioxide (1g) in ether (10ml) according to the method described in Example 2 to give

the title compound (0.9g) whose NMR spectrum was fully consistent with the proposed structure.

EXAMPLE 24

4a-Hydroxy-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4a-t-Butyldimethylsilyloxy-5-oxo-25-cyclohexyl-22,23-dihydro avermectin B1 monosaccharide (0.9g) was dissolved in a methanol-dioxan (1:1 mixture , 36ml) and hydroxylamine hydrochloride (0.9g) dissolved in water (18ml) added. This reaction mixture was stirred and heated at 40°C for 1 hour then concentrated to approximately 20ml by evaporation under vacuum and the product extracted with diethyl ether (2 x 50ml). The combined extracts were washed with aqueous sodium bicarbonate solution (10%, 30ml), water (50ml), dried over anhydrous sodium sulphate and evaporated under vacuum to yield the crude product. This material was chromatographed on silica (Kieselgel, 60, 230-400 mesh, Merck) (50g) eluting with dichloromethane-methanol gradient 99:1 to 96:4 collecting 50ml fractions over 1.5 hours. Fractions 50-54 were combined and evaporated under vacuum to give material which was further purified by high pressure liquid chromatography on an Dynamax (trade mark) column (24 x 250mm; 5µm, ODS-silica, Rainin) eluting with methanol-water 80:20 at 20ml per minute. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 254'-Methyl-5-O-t-butyldimethylsilyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-Oxo-5-O-t-butyldimethylsilyl-25-cyclohexyl-22,23-dihydroavermectin B1-monosaccharide (Example 16) (174mg) was dissolved in dry ether (15ml) and cooled to 0°C under a nitrogen atmosphere. To this solution was added a solution of methyl magnesium bromide (115µl, 3M in diethyl ether) dropwise, and stirring continued for a further 1 hour. After quenching, by adding an aqueous solution of ammonium chloride, (10ml, 10%), the organic layer was separated, washed with water (2 x 10ml), dried over anhydrous sodium sulphate and evaporated under vacuum to give the crude product. This material was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (20g) eluting with dichloromethane-ethyl acetate 9:1. Combining appropriate fractions, and evaporation under vacuum afforded the product which was used without further purification.

EXAMPLE 264'-Methyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-Methyl-5-O-t-butyldimethylsilyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (74mg) (Example 25) was dissolved in methanol (20ml) containing p-toluenesulphonic acid (36mg) and stirred at ambient temperature for 2 hours. After this time solid potassium bicarbonate (50mg) was added and the mixture diluted with water (20ml) and extracted with diethyl ether (2 x 20ml). The combined extracts were washed with water (20ml), saturated aqueous sodium chloride solution (10ml), dried

over anhydrous sodium sulphate and evaporated under vacuum to yield a crude product. This material was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (10g) eluting with dichloromethane-ethyl acetate 4:1. Appropriate fractions were combined and evaporated under vacuum to give the product which was further purified by preparative high pressure liquid chromatography on an Ultrasphere (trade mark) column (10 x 250mm, 5µm, ODS-silica, Beckmann) eluting with methanol-water 85:15 at 5ml per minute. Appropriate fractions were combined and evaporated under vacuum to yield the title product as a white powder. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 27

4'-Methyl-5-oxo-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-Methyl-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (255mg) was oxidised with activated manganese dioxide (250mg) in diethyl ether (50ml) according to the procedure described in Example 2 to give the title compound (208mg) whose NMR spectrum was fully consistent with the proposed structure.

EXAMPLE 28

4'-Methyl-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a solution of 4'-methyl-5-oxo-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (208mg) in a mixture of methanol:dioxan 1:1 (40ml) was added a solution of hydroxylamine hydrochloride (416mg)

- 32 -

dissolved in water (10ml). This mixture was stirred at room temperature and a further amount of hydroxylamine hydrochloride (208mg) was added after 3 hr and stirring continued at 50°C for 5 hr before quenching with the addition of saturated aqueous potassium bicarbonate solution (20ml) and diethyl ether (50ml). The organic extract was washed sequentially with water (20ml) and saturated aqueous sodium chloride solution (20ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product. This material was then chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (10g) eluting with dichloromethane: ethyl acetate 4:1. Appropriate fractions were combined and evaporated to give the desired compound which was further purified using high pressure liquid chromatography on a Dynamax (trade mark) column (10 x 250mm, 5µm, Ultrasphere (trade mark) ODS-silica, Beckman) eluting with acetonitrile:methanol:water 63:12:25 at 5ml per minute. Appropriate fractions were combined and evaporated to dryness to yield the title product as a white solid (63mg). Mass and NMR spectra were fully consisted with the proposed structure.

EXAMPLE 29

5-O-t-Butyldimethylsilyloximino-22,23-dihydro-25-cyclohexylavermectin B1 monosaccharide

Method 1. 5-Oximino-22,23-dihydro-25-cyclohexylavermectin B1 monosaccharide (5.93g) (Example 5), t-butyldimethylsilyl chloride (2.11g) and imidazole (1.9g) were dissolved in dichloromethane (20ml) and the mixture stirred at room temperature for 1hr. Further amounts of t-butyldimethylsilyl chloride (2.11g) and

imidazole (1.9g) were then added and the reaction mixture stirred at 40°C for another 0.5hr. The mixture was washed with water (2 x 20ml), saturated aqueous potassium bicarbonate solution (10ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product. Purification was achieved by chromatography on silica (Kieselgel 60, 230-400 mesh, Merck) (250g) eluting with dichloromethane: ethyl acetate 9:1. Appropriate fractions were combined and evaporated to give the title compound as a white powder (4.0g).

Method 2. 22,23-Dihydro-25-cyclohexylavermectin B1 monosaccharide (Example 4) was oxidized to the 5-oxo derivative using manganese dioxide in anhydrous diethyl ether according to a procedure of Example 2. The 5-oxo-22,23-dihydro-25-cyclohexylavermectin B1 monosaccharide (5.0g) was dissolved in dichloromethane (200ml) and O-(t-butyldimethylsilyl)-hydroxylamine (2.5g) and glacial acetic acid (10ml) were added. The reaction mixture was stirred at room temperature for 18 hrs and then washed with water (50ml), saturated aqueous potassium bicarbonate solution (50ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product. Final purification was achieved by chromatography over silica (Kieselgel 60, 230-400 mesh, Merck) (250g) eluting with a gradient of hexane:diethyl ether 2:1 changing to 1:1. Appropriate fractions were combined and evaporated to give the title compound as a white powder (3.9g).

- 34 -

The NMR spectrum of the products from the two methods was fully consistent with the proposed structure.

EXAMPLE 30

4'-Acetyl-amino-4'-deoxy-5-oximino-22,23-dihydro-25-cyclohexylvermectin B1 monosaccharide

To a solution of 5-O-t-Butyldimethylsilyloximino-22,23-dihydro-25-cyclohexyl vermectin B1 monosaccharide (620mg) (Example 29) in dichloromethane (40ml) were added tetrapropylammonium perruthenate (100mg) and N-methylmorpholine N-oxine (600mg). The reaction mixture was stirred at room temperature for 1 hour and then applied to the top of a silica chromatography column (Kieselgel 60, 230-400 mesh, Merck) (30g). Following elution with dichloromethane, appropriate fractions were combined and evaporated to give the 4'-oxo-derivative which was used directly in the next step by dissolution in methanol (10ml), adding ammonium acetate (1.0g) followed by sodium cyanoborohydride portionwise until TLC indicated complete reduction. The solvent was then removed by evaporation under reduced pressure, the residue taken up in dichloromethane (10ml) and triethylamine (500l) and acetic anhydride (200l) were added. After 1 hr TLC indicated that the reaction was complete. The mixture was evaporated under reduced pressure, the residue taken up in methanol (10ml) and solid p-toluenesulphonic acid added until the solution pH was 3.0. After 1 hr TLC indicated that deprotection was complete and the reaction mixture was poured into a mixture of aqueous sodium bicarbonate solution and ether 1:1 (20ml). The organic layer was separated, dried over

anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product. Purification was achieved by high pressure liquid chromatography on an Dynamax (trade mark) column (24 x 250mm, 5µm, ODS-silica, Rainin) eluting with methanol-water 85:15 at 20ml per minute. Appropriate fractions were combined and evaporated under vacuum to give the product which was further purified by high pressure liquid chromatography on an Dynamax (trade mark) column (24 x 250mm, 5µm, ODS-silica, Rainin) eluting with methanol-water 82:18 at 20ml per minute. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder (50mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 31

5-Oximino-25-cyclohexylavermectin B2

25-Cyclohexylavermectin B2 (50g) was oxidised to its 5-oxo-derivative with activated manganese dioxide (2 x 50g) in diethyl ether (500ml) according to the method described in Example 2. Further reaction with hydroxylamine hydrochloride (53g) in an aqueous methanol - dioxan - water mixture (1:1:1) (900ml) was carried out according to the procedure described in Example 28. The title product was isolated from the reaction by pouring into water (500ml) and extracting with diethyl ether (3 x 500ml). The combined organic extracts were dried over anhydrous magnesium sulphate and evaporated to dryness under vacuum to give the product (53g) which was used directly in the next example without further purification.

- 36 -

EXAMPLE 325-Oximino-23-oxo-25-cyclohexylavermectin B2
monosaccharide

A mixture of 5-Oximino-25-cyclohexylavermectin B2 (500mg) and pyridinium dichromate (1.87g) in dimethylformamide (25ml) was stirred at room temperature under a nitrogen atmosphere for 18 hrs. The reaction mixture was then poured into an ice (25g) and (50 ml) mixture and extracted with diethyl ether (2 x 50ml). The combined ether extract was washed with 2N hydrochloric acid (20ml), water (50ml), dried over anhydrous magnesium sulphate and evaporated to dryness under vacuum to give a residue (350mg) which was dissolved in isopropanol (25ml) containing 1% sulphuric acid v/v. After stirring this reaction mixture for 18 hrs under a nitrogen atmosphere it was poured into an ice-water mixture (50ml) and the product extracted with dichloromethane (2 x 30ml). The combined organic extracts were dried over anhydrous magnesium sulphate and evaporated to dryness under vacuum to give the crude product (300mg) which was purified by high pressure liquid chromatography on a Zorbax column (21.2 x 250mm, 8µm, ODS-silica, Rainin) eluting with methanol - water 78:22 at 9ml per minute. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder. Mass and NMR spectra were fully consistent with the proposed structure.

- 37 -

EXAMPLE 335-Oximino-23-methoximino-25-cyclohexylavermectin B2 monosaccharide

To a solution of 5-oximino-23-oxo-25-cyclohexylavermectin B2 monosaccharide (300mg) (Example 32) in dioxan (150ml) containing sodium acetate (320mg) and methoxylamine hydrochloride (370mg) was added glacial acetic acid (10ml). The reaction was stirred at room temperature for 18hrs then poured into water (200ml) and extracted with dichloromethane (2 x 200ml). The combined extracts were washed with saturated aqueous sodium bicarbonate solution (2 x 100ml) and water (100ml), dried over anhydrous magnesium sulphate and evaporated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (10g) eluting with a gradient of hexane:diethyl ether 1:1 changing to 0:1. Appropriate fractions were combined and evaporated to give the product (98mg) which was further purified by high pressure liquid chromatography on a Zorbax column (21.2 x 250mm, 8µm, ODS-silica, Rainin) eluting with methanol - water 82:18 at 9ml per minute. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder (60mg). Mass and NMR spectra were fully consistent with the proposed structure.

-38 -

EXAMPLE 344'-O-Succinoyl-5-O-t-butyldimethylsilyloximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a solution of 5-O-t-butyldimethylsilyloximino-22,23-dihydro-25-cyclohexyl- avermectin B1 monosaccharide (Example 29) (530mg), diisopropylethylamine (770mg) and 4-dimethylaminopyridine (73mg) in dichloromethane (50ml) was added succinic anhydride (3.6g). The resulting suspension was sonicated for 10min and stirred at room temperature for 18hr then washed with water (2 x 10ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (20g) eluting with a gradient of dichloromethane - ethyl acetate 9:1 changing to 4:1. Appropriate fractions were combined and evaporated to give a solid which was slurried with dichloromethane (20ml), filtered and the filtrate evaporated to dryness under vacuum to give the crude product which was further purified by high pressure liquid chromatography on a Dynamax (trade mark) column (41.4 x 250mm, 8µm, ODS-silica, Rainin) eluting at 9ml per minute with a methanol - water 90:10 mixture changing to 95:5 after 40 mins. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder (407mg). Mass and NMR spectra were fully consistent with the proposed structure.

- 39 -

EXAMPLE 354'-O-Succinoyl-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

A solution of 4'-O-succinoyl-5-O-t-butyltrimethylsilyloximino-22,23-dihydro-25-cyclohexyl-avermectin B1 monosaccharide (Example 34) (50mg), and p-toluenesulphonic acid (50mg) in a dioxan - water mixture 10:1 (11ml) was stirred at room temperature for 2hr. This reaction mixture was then quenched by the addition of saturated aqueous potassium bicarbonate solution (5ml) and water (10ml) and the product extracted with diethyl ether (3 x 20ml). The combined organic extracts were washed with water (3 x 10ml), saturated aqueous sodium chloride solution (5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (0.5g) eluting with ethyl acetate. Appropriate fractions were combined and evaporated to give a solid (46mg) which was further purified by high pressure liquid chromatography on a Utrasphere column (10 x 250mm, 5µm, ODS-silica, Beckman) eluting at 5ml per minute with a methanol - water 80:20 mixture changing to 85:15 after 20 mins and to 90:10 after 40 mins. Appropriate fractions were combined and evaporated under vacuum to yield the potassium salt of the title compound as a white powder (32mg). This compound was dissolved in diethyl ether (10ml), washed with an aqueous citric acid solution (20% w/v, 5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the title compound as a white powder (25mg). Mass and NMR spectra were fully consistent with the proposed structure.

- 40 -

EXAMPLE 364'-O-(3-Methoxycarbonylpropanoyl)-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

A solution of 4'-O-succinoyl-5-O-t-butylldimethylsilyloximino-22,23-dihydro-25-cyclohexyl-avermectin B1 monosaccharide (Example 34) (50mg), and p-toluenesulphonic acid (50mg) in methanol (10ml) was stirred at room temperature for 1hr. This reaction mixture was then quenched by the addition of saturated aqueous potassium bicarbonate solution (2ml) and water (10ml) and the product extracted with diethyl ether (3 x 10ml). The combined organic extracts were washed with water (3 x 5ml), saturated aqueous sodium chloride solution (2 x 5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (1g) eluting with dichloromethane - ethyl acetate 9:1. Appropriate fractions were combined and evaporated to give the title compound as a white powder. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 374'-O-3-(4-Methylpiperazin-1-yl)carbonylpropanoyl)-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a solution of 4'-O-succinoyl-5-O-t-butylldimethylsilyloximino-22,23-dihydro-25-cyclohexyl-avermectin B1 monosaccharide (Example 34) (100mg), 1-hydroxybenzotriazole (15mg) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (24mg) in dry N,N-dimethylformamide (5ml) was added and N-methylpiperazine (11mg) and the mixture stirred at room temperature for 18hr. After pouring into water (20ml) the

- 41 -

product was extracted with diethyl ether (3 x 10ml). The combined extracts were washed with water (3 x 5ml), saturated aqueous sodium chloride solution (2 x 5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give a material which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (10g) eluting with dichloromethane - methanol 95:5. Appropriate fractions were combined and evaporated to give a material (85mg) which was dissolved in methanol (10ml) containing p-toluenesulphonic acid (85mg) and the whole stirred at room temperature for 1hr. This reaction mixture was quenched by adding saturated aqueous potassium bicarbonate (5ml) and water (20ml) and the product extracted with diethyl ether (3 x 10ml). The combined organic extracts were washed with water (3 x 5ml), saturated aqueous sodium chloride solution (2 x 5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (10g) eluting with dichloromethane - methanol 95:5. Appropriate fractions were combined and evaporated to give the title compound as a white powder (52mg). Mass and NMR spectra were fully consistent with the proposed structure.

- 42 -

EXAMPLE 384'-O-(3-(Pyrid-4-ylamino)carbonylpropanoyl)-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a solution of 4'-O-succinoyl-5-O-t-butylldimethylsilyloximino-22,23-dihydro -25-cyclohexyl-avermectin B1 monosaccharide (Example 34) (100mg), 1-hydroxybenzotriazole (30mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (48mg) and diisopropylethylamine (39mg) in dry N,N-dimethylformamide (5ml) was added 4-aminopyridine (21mg) and the mixture stirred at room temperature for 18hr. After pouring into ethyl acetate (20ml) the resulting solution was washed with water (3 x 10ml), saturated aqueous sodium chloride solution (2 x 5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give a material (90mg) which was dissolved in methanol (10ml) containing p-toluenesulphonic acid (20mg) and the whole stirred at room temperature for 1hr. This reaction mixture was quenched by adding saturated aqueous potassium bicarbonate (5ml) and water (20ml) and the product extracted with diethyl ether (3 x 10ml). The combined organic extracts were washed with water (3 x 5ml), saturated aqueous sodium chloride solution (2 x 5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (5g) eluting with ethyl acetate. Appropriate fractions were combined and evaporated to dryness under vacuum to give the title compound as a white powder (29mg). Mass and NMR spectra were fully consistent with the proposed structure.

- 43 -

EXAMPLE 394'-O-(N,N'-bis-(9-fluorenylmethoxycarbonyl)lysiny)-5-O-t -butyldimethylsilyloximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a solution of N,N'-bis-(9-fluorenylmethoxycarbonyl)lysine (1.32g) in dichloromethane (250ml) was added dicyclohexylcarbodiimide (230mg) and the mixture stirred at room temperature for 1 hr. The resulting suspension was then sonicated for 0.5hr then filtered through a layer of Hyflo (trade mark) and the filtrate concentrated under reduced pressure to a volume of approximately 100 ml to give a solution of the desired N,N'-bis-(9-fluorenylmethoxycarbonyl)lysine anhydride. To this solution was added 5-O-t-butyldimethylsilyloximino-22,23-dihydro-25-cyclohexyl- avermectin B1 monosaccharide (Example 29) (670mg), diisopropylethylamine (294mg) and 4-dimethylaminopyridine (185mg). The reaction mixture was stirred at room temperature for 90hrs then concentrated to dryness under reduced pressure to give a residue which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (200g) eluting with dichloromethane changing to dichloromethane - ethyl acetate 9:1 after 11 had been collected. Appropriate fractions were combined and evaporated to dryness under vacuum to give the title compound as a white powder (850mg). The NMR spectrum was fully consistent with the proposed structure.

- 44 -

EXAMPLE 40

4'-O-(N,N'-bis-(9-Fluorenylmethoxycarbonyl)lysiny1)-5-ox imino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-O-(N,N'-bis-(9-fluorenylmethoxycarbonyl)lysiny1)-5-O-t-butyldimethylsilyloximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (850mg) (Example 39) was dissolved in methanol (20ml) containing p-toluenesulphonic acid (20mg) and the whole stirred at room temperature for 2hrs. This reaction mixture was quenched by adding saturated aqueous potassium bicarbonate (5ml) and water (20ml) and the product extracted with diethyl ether (3 x 10ml). The combined organic extracts were washed with water (3 x 5ml), saturated aqueous sodium chloride solution (2 x 5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (25g) eluting with dichloromethane - ethyl acetate 9:1 changing to 4:1 after 200ml of eluate had been collected. Appropriate fractions were combined and evaporated to dryness under vacuum to give the title compound as a white powder (274mg). The NMR spectrum was fully consistent with the proposed structure.

- 45 -

EXAMPLE 41

4'-O-Lysinyl-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4'-O-(N,N'-bis-(9-fluorenylmethoxycarbonyl)lysiny)-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (274mg) (Example 40) was dissolved in acetonitrile (30ml) containing piperidine (150mg) and the mixture stirred at room temperature for 8 hrs. This was concentrated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (25g) eluting with dichloromethane - methanol - 0.880 ammonia solution 80:20:1. Appropriate fractions were combined and evaporated to dryness under vacuum to give the title compound which was freeze dried from t-butanol to yield a white powder (157mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 42

5-(Trimethylacetyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a stirred solution of 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (50mg) in dichloromethane (2ml) at room temperature was added triethylamine (72l) followed by trimethylacetyl chloride (80l). After leaving to stand for 18 hrs an aqueous citric acid solution (10% w/v, 2ml) was added and the organic layer separated, washed with saturated aqueous sodium chloride solution (2ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product which was chromatographed on silica (Kieselgel 60, 230-400 mesh,

- 46 -

Merck) (5g) eluting with diethyl ether. Appropriate fractions were combined and evaporated to dryness under vacuum to give a material (53mg) which was further purified by high pressure liquid chromatography on a Dynamax (trade mark) column (21.2 x 250mm, 5µm, ODS-silica, Rainin) eluting at 20ml per minute with a methanol - water 95:5 mixture. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder (18mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 43

5-(Benzoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (70mg) in dichloromethane (30ml) was reacted with triethylamine (50l) and benzoyl chloride (100l) and the desired product extracted in a manner identical to that described in Example 42. Purification was achieved by high pressure liquid chromatography on a Dynamax (trade mark) column (41.4 x 250mm, 8µm, ODS-silica, Rainin) eluting at 45ml per minute with a methanol - water 90:10 mixture. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder (28mg). Mass and NMR spectra were fully consistent with the proposed structure.

- 47 -

EXAMPLE 445-(N-Methylcarbamoyloximino)-25-cyclohexyl-22,23-dihydroa vermectin B1 monosaccharide

To a stirred solution of 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (106mg) in dichloromethane (10ml) was added methyl isocyanate (15l) and the mixture stirred for 1hr. A further amount of methyl isocyanate (30l) was then added and the reaction stirred for another 72 hrs before adding saturated aqueous sodium chloride solution (10ml) and ether (30ml). The organic extract was dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product (150mg) which was purified by high pressure liquid chromatography on a Dynamax (trade mark) column (41.4 x 250mm, 8µm, ODS-silica, Rainin) eluting at 45ml per minute with a methanol - water 91:9 mixture. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder (80mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 455-(N,N-Dimethylcarbamoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a stirred solution of 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (50mg) in dichloromethane (2ml) at room temperature was added triethylamine (72l) and 4-dimethylaminopyridine (1mg) followed by N,N-dimethylcarbamoyl chloride (58l). After 3hrs further N,N-dimethylcarbamoyl chloride (58l) was added and the reaction left to stand for 18 hrs. An aqueous citric acid solution (10% w/v, 2ml) and diethyl ether (20ml) were then added and the organic layer

- 48 -

separated, washed with saturated aqueous sodium chloride solution (5ml), dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to give the crude product which was purified by high pressure liquid chromatography on a Dynamax (trade mark) column (21.2 x 250mm, 5µm, ODS-silica, Rainin) eluting at 10ml per minute with a methanol - water 90:10 mixture. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder (18mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 46

5-(4-Methylpiperazinyl-1-carboxyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a stirred solution of N-methylpiperazine (0.65ml) and triethylamine (1.3ml) in toluene (25ml) at 0°C was added dropwise a solution of phosgene in toluene (20% , 5.1ml) over a period of 15 min. The reaction was allowed to warm to room temperature, stirred for 3hr, filtered and concentrated to approximately 10ml under reduced pressure to give a solution of 1-chlorocarbonyl-4-methylpiperazine which was reacted with 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (300mg), triethylamine (110l) and 4-dimethylaminopyridine (5mg) in dichloromethane (10ml) at room temperature according to the method described in Example 45. Purification of the desired material was achieved by chromatography on silica (Kieselgel 60, 230-400 mesh, Merck) (35g) eluting with dichloromethane. Appropriate fractions were combined and evaporated to dryness under vacuum to give a material (53mg) which was further purified by high pressure liquid chromatography

on a Dynamax (trade mark) column (21.2 x 250mm, 5µm, ODS-silica, Rainin) eluting at 20ml per minute with a methanol - water 95:5 mixture. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 47

5-(t-Butyloxycarbonyloximino)-25-cyclohexyl-22,23-dihydro avermectin B1 monosaccharide

To a stirred solution of 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (60mg) and triethylamine (50l) in dichloromethane (5ml) at room temperature was added t-butyloxycarbonyl anhydride (60mg). After allowing to stand for 48hrs the reaction was evaporated to dryness under vacuum to give a residue which was dissolved in dichloromethane and chromatographed on silica (Kieselgel 60, 230-400 mesh, Merck) (5g) eluting with dichloromethane. Appropriate fractions were combined and evaporated to dryness under vacuum to give the title compound as a white powder (45mg). Mass and NMR spectra were fully consistent with the proposed structure.

- 50 -

EXAMPLE 485-(N-(4-Formylphenyl)-carbamoyloximino)-25-cyclohexyl-22, 23-dihydroavermectin B1 monosaccharide

4-Formylphenylisocyanate was prepared according to the method described in J. Med. Chem., 32(10), 2354, (1989) and was reacted with 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (500mg), in dry dichloromethane (50ml) at room temperature for 1hr according to the method described in Example 43. Purification of the desired material was achieved using chromatography over silica (Kieselgel 60, 230-400 mesh, Merck) (125g) eluting with a gradient of hexane - ether 1:1 changing to 20:80. Appropriate fractions were combined and evaporated to dryness under vacuum to give the title compound as a white powder (300mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 495-(N-(4-(Diethylaminomethyl)phenyl)-carbamoyloximino)-25 -cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4-Diethylaminomethylbenzoyl chloride was prepared according to the method described in United States Patent publication US-4623486 and was reacted with 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (100mg), in dry dichloromethane (50ml) containing triethylamine (450l) and 4-dimethylaminopyridine (126mg) at room temperature for 1hr according to the method described in Example 45. Purification of the desired material was achieved using chromatography over silica (Kieselgel 60, 230-400 mesh, Merck) (5g) eluting with a gradient of methanol -

- 51 -

dichloromethane 0:100 changing to 10:90.

Appropriate fractions were combined and evaporated to dryness under vacuum to give the title compound as a white powder (11mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 50

5-(N-(4-(4-Methyl-1-piperazinyl-methyl)phenyl)-carbamoyl oximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

4-(4-Methylpiperazin-1-ylmethyl)benzoyl chloride was prepared according to the method described in United States Patent publication US-4623486 and was reacted with 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) in a manner identical to that described in Example 48. The title compound was obtained as a white powder (18mg). Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 51

5-(N-(3-Pyridylcarbonyl)-carbamoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide

To a stirred solution of nicotinamide (4.88g) in dry 1,2-dichloroethane (500ml) was added dropwise oxalyl chloride (5.24ml). The mixture was heated under reflux for 4.5h then cooled, filtered and the resulting solution containing nicotinoyl isocyanate (50ml) was reacted with 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (500mg), in dichloromethane (10ml) at room temperature. After allowing to stand for 18hr further nicotinoyl isocyanate solution (25ml) was added and the mixture left at room temperature for a further 18hr before evaporating to dryness under vacuum

to give a residue which was purified by high pressure liquid chromatography on a Dynamax (trade mark) column (41.4 x 250mm, 8µm, ODS-silica, Rainin) eluting at 45ml per minute with a methanol - acetonitrile - water 20:65:15 mixture. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder. Mass and NMR spectra were fully consistent with the proposed structure.

EXAMPLE 52

5-(N-(3-Pyridyl)-carbamoyloximino)-25-cyclohexyl-22,23-di hydroavermectin B1 monosaccharide

To a solution of nicotinic acid hydrazide dihydrochloride (2g) in water (10ml) was added a solution of sodium nitrite (1.6g) in water (10ml), keeping the temperature below 20°C. Diethyl ether (50ml) was then added and the mixture basified by the careful addition of solid sodium bicarbonate. The organic layer was separated, washed with water (20ml), dried over anhydrous magnesium sulphate and evaporated to dryness under vacuum to give nicotinyl azide (1.1g) m.pt. 54°C. This azide (1.1g) was stirred in dry toluene (10ml) and heated at 100°C under a nitrogen atmosphere for 8hr to give a solution containing 3-pyridyl isocyanate. A portion of this solution (1ml) was reacted with 5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide (Example 5) (100mg), in toluene (10ml) at room temperature for 1hr before pouring into a diethyl ether - water mixture (1:1, 30ml). The organic layer was separated, dried over anhydrous magnesium sulphate and evaporated to dryness under vacuum to give a residue (130mg) which was purified by high pressure liquid

- 53 -

chromatography on a Dynamax (trade mark) column (41.4 x 250mm, 8µm, ODS-silica, Rainin) eluting at 45ml per minute with a methanol - water 85:15 mixture, changing to 87:13 after 15 mins. Appropriate fractions were combined and evaporated under vacuum to yield the title compound as a white powder (52mg). Mass and NMR spectra were fully consistent with the proposed structure.

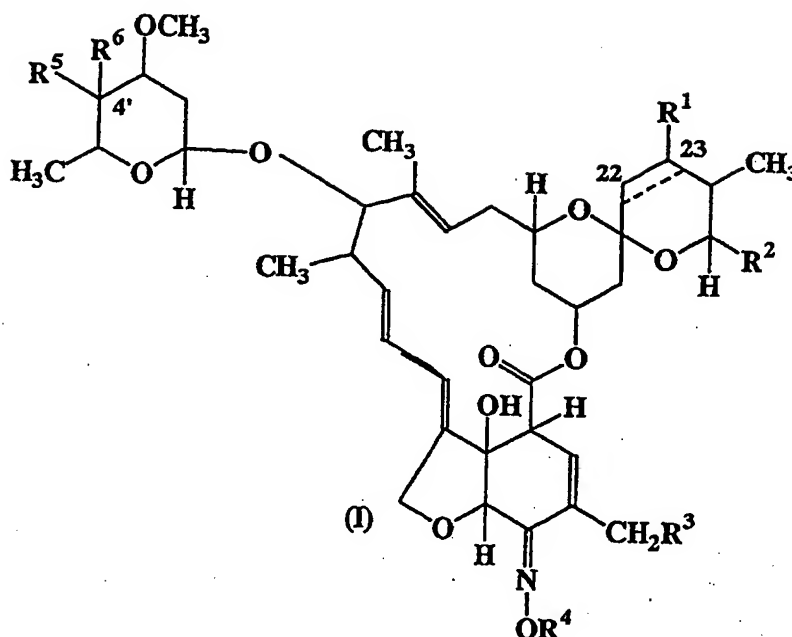
EXAMPLE 53

5-(-Allylcarbamoyloximino)-25-cyclohexyl-22,23-dihydroa vermetin B1 monosaccharide

5-oximino-25-cyclohexyl-22,23-dihydroa vermetin B1 monosaccharide (Example 5) (500mg) was reacted with allyl isocyanate (108mg) in dichloromethane (50ml) according to the method described in Example 43 to give the title compound as a white powder (352mg). Mass and NMR spectra were fully consistent with the proposed structure.

CLAIMS

1. A compound of formula (I).



wherein the broken line at the 22-23 position represents an optional bond and either this bond is present and R^1 is absent or this bond is absent and R^1 is H, OH, oxo or oximino optionally substituted by a C_1 - C_6 alkyl group, R^2 is a C_1 - C_6 alkyl, C_2 - C_6 alkenyl or C_3 - C_6 cycloalkyl group, or a 3- to 6- membered heterocyclic ring containing a sulphur or oxygen atom, said ring being saturated or fully or partially unsaturated and optionally substituted by one or more C_1 - C_6 alkyl groups or halogen atoms, R^3 is H or OH, and R^4 is H or a group capable of being hydrolysed in vivo to yield a compound in which R^4 is H, R^5 is OH, optionally substituted with a group capable of being hydrolysed in vivo to yield a compound in which R^5 is OH, and R^6 is H or C_1 - C_6 alkyl or R^6 is H and R^5 is amino, optionally substituted with at least one group selected from C_1 - C_6 alkyl and acyl groups.

2. A compound according to Claim 1, in which said groups capable of being hydrolysed in vivo are independently substituted or unsubstituted C₂-C₆ alkanoyl, aroyl, carbamoyl, C₁-C₆ alkoxy carbonyl, dicarboxylic acid or amino acid group.
3. A compound according to Claim 2, in which said group capable of being hydrolysed in vivo is an acetyl, t-butylcarbonyl, t-butylloxycarbonyl, benzoyl, methylpiperazinecarbonyl, N-methylcarbamoyl, N,N-dimethylcarbamoyl, formylphenylcarbamoyl, N-(4-diethylaminomethylphenyl)-carbamoyl, N-(4-methyl-1-piperazin-methylphenyl)-carbamoyl, N-(3-pyridylcarbonyl)-carbamoyl, N-(3-pyridyl)-carbamoyl, allylcarbamoyl, succinoyl, methoxysuccinoyl, 4-methylpiperazinesuccinoyl, pyrid-4-ylaminosuccinoyl, lysinyl or a N, N¹-bis(9-fluorenylmethoxycarbonyl) lysinyl group.
4. A compound according to Claim 1, 2 or 3 in which R² is an alkyl or cycloalkyl group.
5. A compound according to Claim 4, in which R² is cyclohexyl, isopropyl or sec-butyl.
6. A compound according to any preceding claim, in which R³ is H and the optional bond at the 22-23 position is present or this optional bond is absent and R¹ is H or OH.
7. A compound according to claim 1 which R³ and R⁴ are H.
8. Any one of the following compounds:
 - 5-oximino-22,23-dihydroavermectin B1a monosaccharide,
 - 5-oximino-22,23-dihydro-25-cyclohexylavermectin B1 monosaccharide,
 - 5-oximino-25-cyclohexylavermectin B2 monosaccharide,
 - 5-oximino-25-cyclohexylavermectin B1 monosaccharide,
 - 4'-epi-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide,
 - 4a-hydroxy-5-oximino-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide,
 - 5-oximino-25-(4-tetrahydropyranyl)-22,23-

dihydroavermectin B1 monosaccharide.
4'-O-Acetyl-5-oximino-25-cyclohexyl-22,23-
dihydroavermectin B1 monosaccharide,
4'-methyl-5-oximino-25-cyclohexyl-22,23-dihydroavermectin
B1 monosaccharide,
4'-acetylamino-4'-deoxy-5-oximino-22,23-dihydro-25-
cyclohexylavermectin B1 monosaccharide,
5-oximino-23-oxo-25-cyclohexylavermectin B2
monosaccharide,
5-oximino-23-methoximino-25-cyclohexylavermectin B2
monosaccharide,
4'-O-succinoyl-5-oximino-25-cyclohexyl-22,23-
dihydroavermectin B1 monosaccharide,
4'-O-(3-methoxycarbonylpropanoyl)-5-oximino-25-cyclohexyl-
22,23-dihydroavermectin B1 monosaccharide,
4'-O-(3-(4-methylpiperazin-1-yl)carbonylpropanoyl)-5-
oximino-25-cyclohexyl-22,23-dihydroavermectin B1
monosaccharide,
4'-O-(3-(pyrid-4-ylamino)carbonylpropanoyl)-5-oximino-25-
cyclohexyl-22,23-dihydroavermectin B1 monosaccharide,
4'-O-(N,N'-bis-(9-fluorenylmethoxycarbonyl)lysiny)-5-
oximino-25-cyclohexyl-22,23-dihydroavermectin B1
monosaccharide,
4'-O-lysiny-5-oximino-25-cyclohexyl-22,23-
dihydroavermectin B1 monosaccharide,
5-(trimethylacetyloximino)-25-cyclohexyl-22,23-
dihydroavermectin B1 monosaccharide,
5-(benzoyloximino)-25-cyclohexyl-22,23-dihydroavermectin
B1 monosaccharide,
5-(N-methylcarbamoyloximino)-25-cyclohexyl-22,23-
dihydroavermectin B1 monosaccharide,
5-(N,N-dimethylcarbamoyloximino)-25-cyclohexyl-22,23-
dihydroavermectin B1 monosaccharide,
5-(4-methylpiperazinyl-1-carbonyloximino)-25-cyclohexyl-
22,23-dihydroavermectin B1 monosaccharide,
5-(t-butyloxycarbonyloximino)-25-cyclohexyl-22,23-
dihydroavermectin B1 monosaccharide,

5-(N-(4-formylphenyl)-carbamoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide,
5-(N-(4-(diethylaminomethyl)phenyl)-carbamoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide,
5-(N-(4-(4-methyl-1-piperazinyl-methyl)phenyl)-carbamoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide,
5-(N-(3-pyridylcarbonyl)-carbamoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide,
5-(N-(3-pyridyl)-carbamoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide,
5-(N-allylcarbamoyloximino)-25-cyclohexyl-22,23-dihydroavermectin B1 monosaccharide.

9. A pharmaceutical or veterinary composition, comprising a compound according to any preceding claim and a pharmaceutically acceptable carrier or excipient.

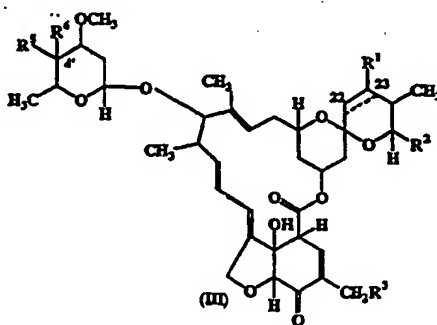
10. A compound according to any one of claims 1 to 8 for use in animal or human medicine.

11. A compound according to any one of claims 1 to 8, for use as an antiparasitic agent.

12. Use of a compound according to any one of claims 1 to 8 for making a medicament for treatment or prophylaxis of flea infestations.

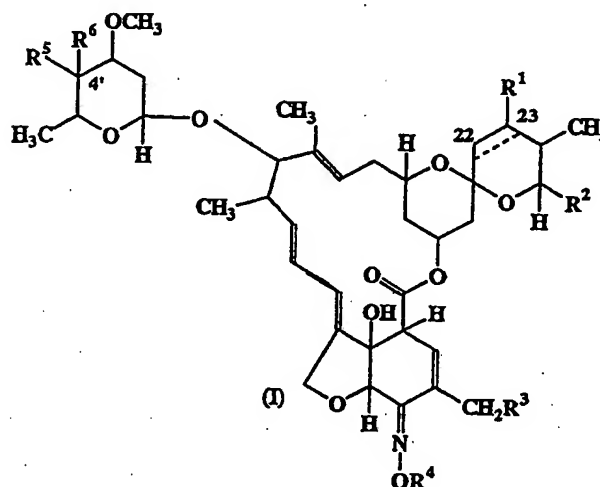
13. A method of treatment or prophylaxis of parasitic infections, which comprises administering to an animal or human an effective amount of a compound according to any one of claims 1 to 8.

14. A compound of formula (III).



wherein the broken line, R^1 , R^2 , R^3 and R^6 are as defined in claim 1, or R^5 is α -oleandrosyloxy and R^6 is H.

15. A process for preparing a compound of formula (I):



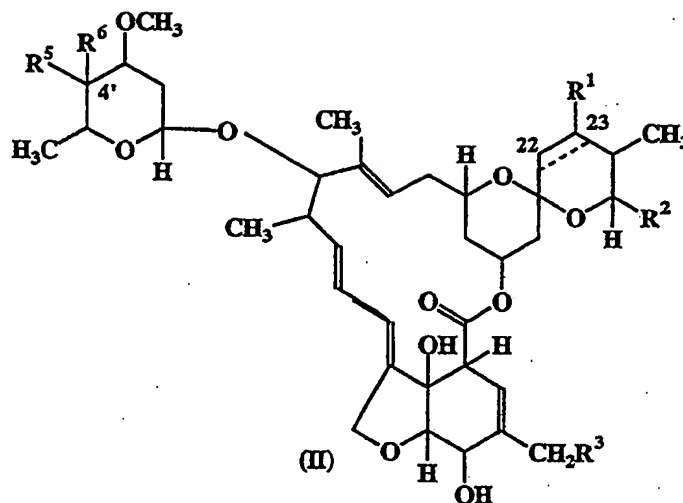
wherein the broken line at the 22-23 position represents an optional bond and either this bond is present and R^1 is absent or this bond is absent and R^1 is H, OH, oxo or oximino optionally substituted by a C_1 - C_6 alkyl group, R^2 is a C_1 - C_6 alkyl, C_2 - C_6 alkenyl or C_3 - C_6 cycloalkyl group, or a 3- to 6- membered heterocyclic ring containing a sulphur or oxygen atom, said ring being saturated or fully or partially unsaturated and which may optionally be substituted by one or more C_1 - C_6 alkyl groups or halogen atoms,

R^3 is H or OH,

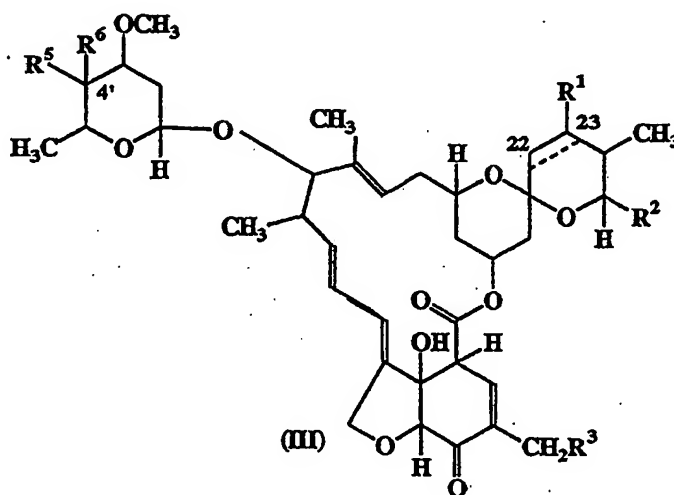
R^4 is H or a group capable of being hydrolysed in vivo to yield a compound in which R^4 is H,

R^5 is OH, optionally substituted with a group capable of being hydrolysed in vivo to yield a compound in which R^5 is OH, and R^6 is H or C_1 - C_6 alkyl or R^6 is H and R^5 is amino, optionally substituted with at least one group selected from C_1 - C_6 alkyl and acyl groups.

which comprises the steps (1) of oxidising a compound of formula (II):



wherein the broken line, R^1 , R^2 , R^3 and R^6 are as defined above and R^5 is as defined above or R^5 is 2- α -oleandrosyloxy and R^6 is H to yield a compound of formula (III):




- and (ii) allowing the compound of formula (III) to react with a compound of formula R^4-O-NH_2 , where R^4 is as defined above
- and where R^5 is α -oleandrosyloxy, hydrolysing the compound obtained to yield a compound of formula (I),
- and (iii) if necessary replacing group R^4 when the latter is H with said group capable of being hydrolysed in vivo to yield a compound in which R^4 is H,
- if necessary, the process further comprising one or more of the following steps before or after steps (i), (ii) and (iii),
- (iv) substituting group R^5 when the latter is OH with said group capable of being hydrolysed in vivo to yield a compound in which R^5 is OH,
- (v) oxidising group R^1 when the latter is OH to oxo,
- (vi) reacting the compound obtained from step (v) with hydroxylamine optionally substituted by a C_1-C_4 alkyl group to yield a compound in which R^1 is optionally substituted oxo,
- (vii) hydrogenating the compound to reduce a double bond at the 22-23 position to a single bond,
- (viii) oxidising a compound in which R^3 is H to a compound in which R^3 is OH,
- (ix) oxidising a compound in which R^5 is OH and R^6 is H to a compound in which R^5 is oxo and R^6 is absent, and either:
- (x) reducing the compound obtained from (IX) to produce a compound in which R^5 is an epi-OH group, or
- (xi) reacting the compound obtained from (IX) with a Grignard reagent to yield a compound in which R^5 is OH and R^6 is alkyl, or
- (xii) subjecting the compound obtained from (IX) to reductive amination to yield a compound in which R^5 is an amino or alkylamine group, and if necessary acylating the compound obtained, any free OH groups being protected if necessary during any of the above steps.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 94/00095

A. CLASSIFICATION OF SUBJECT MATTER		
IPC6: C07H 19/01, C07H 17/08, A61K 31/70 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC6: C07H, A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
STN, CA		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A1, 0519731 (MERCK & CO. INC.), 23 December 1992 (23.12.92) --	1-12, 14-15
X	EP, A1, 0506331 (MERCK & CO. INC.), 30 Sept 1992 (30.09.92) --	1-12, 14-15
X	EP, A2, 0411897 (MERCK & CO. INC.), 6 February 1991 (06.02.91) --	1-12, 14-15
X	EP, A2, 0428286 (MERCK & CO. INC.), 22 May 1991 (22.05.91) --	1-12, 14-15
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
27 April 1994		18.05.94
Name and mailing address of the ISA/  European Patent Office, P.B. 3818 Patendlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 631 epo nl, Fax (+31-70) 340-3016		Authorized officer EVA JOHANSSON

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 94/00095

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A2, 0379341 (MERCK & CO. INC.), 25 July 1990 (25.07.90) --	1-12
X	EP, A2, 0480693 (MERCK & CO. INC.), 15 April 1992 (15.04.92) --	1-12
A	EP, A1, 0535734 (MERCK & CO. INC.), 7 April 1993 (07.04.93) --	1-12
A	EP, A1, 0428285 (MERCK & CO. INC.), 22 May 1991 (22.05.91) -- -----	1-12, 15

INTERNATIONAL SEARCH REPORT

SA 85009

Information on patent family members

26/02/94

International application No.

PCT/EP 94/00095

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0519731	23/12/92	AU-A- 1844892 CA-A- 2071803 US-A- 5262400	24/12/92 21/12/92 16/11/93
EP-A1- 0506331	30/09/92	AU-B- 643603 AU-A- 1387192 JP-A- 5163277 US-A- 5208222	18/11/93 01/10/92 29/06/93 04/05/93
EP-A2- 0411897	06/02/91	AU-B- 630992 AU-A- 5998890 CA-A- 2022113 JP-A- 3141291 US-A- 5023241	12/11/92 31/01/91 01/02/91 17/06/91 11/06/91
EP-A2- 0428286	22/05/91	AU-B- 640421 AU-A- 6557090 CA-A- 2028763 JP-A- 3218382	26/08/93 02/05/91 01/05/91 25/09/91
EP-A2- 0379341	25/07/90	AU-B- 618446 AU-A- 4856790 CA-A- 2008150 JP-A- 2270879 US-A- 5015630	19/12/91 26/07/90 19/07/90 05/11/90 14/05/91
EP-A2- 0480693	15/04/92	AU-A- 8574691 JP-A- 5202057	16/04/92 10/08/93
EP-A1- 0535734	07/04/93	AU-A- 2603792	01/04/93
EP-A1- 0428285	22/05/91	AU-B- 632380 AU-A- 6557290 CA-A- 2028603 JP-A- 3209392 US-A- 5055454	24/12/92 02/05/91 01/05/91 12/09/91 08/10/91